VIROLOGY 216, 444–450 (1996)
ARTICLE NO. 0082

SHORT COMMUNICATION

mulatta) against the Infection with SIVmac32H Grown on T-Cells or Derived *ex Vivo*

WOLFGANG LÜKE,[1] CHEICK COULIBALY, ULF DITTMER, GERALD VOSS, REINHARD OESTERLE, BIRGIT MAKOSCHEY, ULRIKE SAUERMANN, ELKE JURKIEWICZ, CHRISTIANE STAHL-HENNIG, HARALD PETRY, AND GERHARD HUNSMANN

Department of Virology and Immunology, German Primate Centre, Kellnerweg 4, D-37077 Göttingen, Germany

Received October 2, 1995; accepted December 8, 1995

The efficacy of three SIVmac32H gp130 vaccines was compared in rhesus monkeys. Three rhesus monkeys were each immunized over a period of 20 weeks with a total of 600 μg virion-derived gp130 oligomers (O-gp130) mixed with keyhole limpet hemocyanin and emulsified with incomplete Freund's adjuvant. Three other monkeys were infected with 5×10^8 PFU of vaccinia virus wild type (VV-wt) while three additional animals received an equivalent dose of VV expressing the gp130 of SIVmac (VV-gp130). At Week 8, the two VV-wt animals received an additional immunization with 100 μg O-gp130 each. All VV-infected animals then received booster immunizations at Weeks 12, 16, and 20 with a total of 300 μg O-gp130 per animal. All animals along with two controls were challenged iv with 50 MID_{50} of T-cell-grown SIVmac32H at Week 22. Four weeks after the challenge and thereafter, both controls and one animal from either VV group were infected as demonstrated by polymerase chain reaction (PCR), virus isolation, and antibody response. In contrast, all O-gp130 animals and one animal each from the VV-wt and the VV-gp130 group were completely protected as shown by negative PCR and virus reisolation. One animal of the VV-gp130 group was partially protected, since it remained virus isolation negative but became PCR positive. All protected animals did not develop a secondary antibody response. Six months after the first challenge, the five completely protected animals were reimmunized twice 4 weeks apart with a total of 200 μg O-gp130 per animal. Two weeks later, all animals were challenged with 5 MID_{50} of the SIVmac32H/spI prepared from the spleen of an immunized, but unprotected SIV-infected rhesus monkey. After the second challenge, all three control animals and one of the vaccinees became productively infected. In contrast, two animals were completely protected, one from the former O-gp130 and one from the former VV-gp130 group. One animal from the former VV-wt group was only DNA–PCR positive and thus partially protected. Therefore, immunization with virion-derived gp130 oligomers of SIVmac32H can confer protection against the infection with T-cell-grown SIVmac32H as well as the *ex vivo* isolate SIVmac32H/spI.

Three types of animal models have been developed to examine potential AIDS vaccines. Chimpanzees are infectable with HIV-1 (*1*), whereas macaques replicate simian immunodeficiency virus (SIV) (*2*) and HIV-2 (*3*). However, only SIV-infected macaques develop a disease comparable to human AIDS (*4*). Whole inactivated virus (WIV), inactivated cell-bound antigen, detergent-treated virus, a split vaccine like Tween–ether (TE) extracts, and ISCOMs can protect macaques from infection by SIV and HIV-2 (*5–8*). Interestingly, WIV of HIV-1 was not protective in chimpanzees (*9*).

Subunit vaccines were tested in all three animal models. In early trials with chimpanzees protection could not be induced with virion-derived or recombinant gp120 (*10, 11*). However, in subsequent experiments a protective immunity was induced with recombinant gp120 (*12*) and an immunization protocol including vaccinia virus (VV) priming and boosting with a mixture of recombinant viral components (*13*). In the HIV-2 macaque model only one experiment with a subunit vaccine was published. Highly purified, virion-derived gp130 oligomers induced a sterilizing immunity against the homologous challenge and a partial protection against a challenge with a heterologous, more replication-competent HIV-2 isolate (*14*). Additional subunit vaccine trials were carried out in the SIV macaque model. With an enriched, virion-derived but not purified monomeric gp130 preparation, 50% protection was obtained against the homologous SIV infection (*15*). In contrast immunity against primary infection with SIVmac could not yet be achieved with recombinant gp130 or gp160 (*16–19*). However, macaques were protected against an SIV challenge after priming

[1] To whom reprint requests should be addressed. Fax: (+49-551) 3851-184.

0042-6822/96 $18.00

with live recombinant VV expressing gp160 followed by immunization with partially purified recombinant gp160 (*20*). Considering these reports, more clear-cut results were desirable.

Here we report on a vaccine trial in nine rhesus macaques (*Macaca mulatta*) immunized with purified, virion-derived SIVmac32H gp130 oligomers. Three animals were primed with VV wild type (VV-wt) and another three with VV expressing the gp130 (VV-gp130). The preparation and *in vivo* titration of the SIVmac32H challenge stock have been described (*21*). For preparation of the SIVmac32H/spI, the spleen of an SIV-infected rhesus monkey (*21*) was homogenized, the homogenate was cleared by low-speed centrifugation, and the supernatant was adjusted to a protein concentration of 5 mg/ml. For *in vivo* titration, two rhesus monkeys per dose were inoculated with 1 ml of a 10^{-2}, 10^{-3}, or 10^{-4} dilution of the virus stock. Infection was monitored by polymerase chain reaction (PCR) (*22, 23*), virus isolation (*23*), and antibody response in an ELISA with recombinant SIVmac gp140 (rgp140) (*24*). Eight weeks after infection and thereafter, all animals infected with the 10^{-2} and 10^{-3} dilutions were PCR positive, virus could be isolated, and a primary immune response had developed with the exception of one animal in the 10^{-3} group (data not shown). All animals of the 10^{-4} group remained virus isolation negative and did not develop a primary immune response despite becoming DNA–PCR positive (data not shown). Thus, 0.5 ml of a 10^{-3} dilution of our SIVmac32H/spI virus stock contained 5 MID_{50}.

For the immunization experiments, the SIVmac32H was propagated in C8166 cells, harvested by differential centrifugation, and the gp130 oligomers were purified by a two-step procedure (*25*). The purity of the preparation was demonstrated by radioimmunoprecipitation assays (RIPA) of ^{125}I-labeled gp130 oligomers with different antisera as well as monoclonal antibodies (MAB). As shown in Fig. 1A, only the antiserum from SIVmac251-infected rhesus monkey 1604 (lane 1) and the antiserum from HIV-2ben-infected cynomolgous monkey 778 (lane 2) reacted with the gp130 oligomers, but neither two HIV-1-specific human sera (lanes 3 and 4) nor a monkey control serum (lane 5). Major contaminating proteins could not be detected by MABs against HLA class I and II (lanes 6 and 7), β2-microglobulin (lane 8) and the CD4 molecule (lane 9). The gp130 oligomers still bound to CD4 (*14*) and thus their structural integrity was retained (Fig. 1B). The gp130–CD4 complexes could be precipitated with the antiserum from rhesus monkey 1604, infected with SIVmac251 (lane 1) and the anti-CD4 MAB OKT4 (lane 4). Precipitates were not obtained with the MAB OKT4a directed against the binding site on the CD4 molecule of the external glycoproteins of immunodeficiency viruses (lane 2) and a monkey control serum (lane 3).

For vaccine preparation, the gp130 oligomers (O-gp130) were mixed with keyhole limpet hemocyanin (Calbiochem, La Jolla, CA) in a ratio of 1:3 and emulsified in incomplete Freund's adjuvant. All animals were derived from the DPZ breeding colony containing only monkeys of Indian origin. Three rhesus monkeys were immunized intramuscularly five times at Weeks 0, 4, 8, 12, and 20 with a single dose of 100 μg O-gp130 per animal. Three other rhesus monkeys were infected by skin scarification with 5×10^8 PFU of the live recombinant VV-gp130 of SIVmacBK28 clone (kindly provided by Dr. A. Burny, University of Brussels, Belgium) at Weeks 0 and 8. At Weeks 12, 16, and 20 these animals were boosted with 100 μg O-gp130 per dose. Two additional macaques were infected with live VV-wt at 0 and 8 weeks and boosted in Weeks 8, 12, 16, and 20 with O-gp130, again with 100 μg per dose. At Week 22, all animals were challenged with 50 MID_{50} of the T-cell-grown SIVmac32H. At the time of the first challenge, all vaccinated animals had generated high gp130 antibody titers during the immunization period (*25*). The gp130 titers of the O-gp130-immunized animals ranged between 10^4 and 10^5 (Table 1) as determined in an ELISA with rgp140 of SIVmac (*24*). The highest anti-gp130 titers were obtained in the animals primed with VV-gp130 and boosted with O-gp130. Their titers ranged between 10^3 and 10^6. However, the highest virus neutralizing antibody titers (*26*) up to 10^4 had developed in the O-gp130-immunized animals, whereas the titers in the VV-gp130-primed animals ranged between 0.8×10^2 and 3.2×10^2. The VV-wt animals developed neither high gp130 binding titers nor neutralizing antibodies. In individual sera high neutralizing antibodies corre-

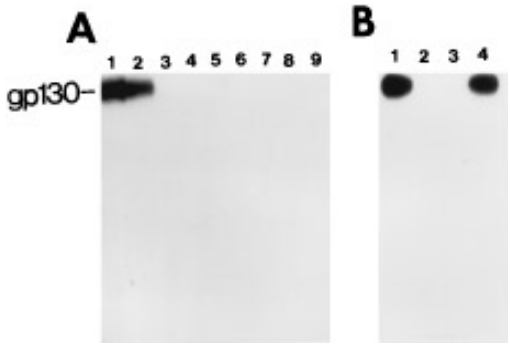

FIG. 1. Serological reactivity and CD4 binding specificity of SIVmac32H gp130 oligomers. ^{125}I-labeled gp130 oligomers were analyzed by SDS–PAGE and autoradiography after immunoprecipitation (A) with various antisera obtained from rhesus monkey 1604, experimentally infected with SIVmac251 (lane 1), from cynomolgus monkey 778 experimentally infected with HIV-2ben (lane 2), from two HIV-1 patients (lane 3 and 4) and a negative control serum (lane 5), as well as after immunoprecipitation with monoclonal antibodies directed against MHC class I (lane 6), MHC class II (lane 7), β2-microglobulin (lane 8), and OKT4 (lane 9). (B) ^{125}I–gp130–CD4 complexes were immunoprecipitated with the antiserum of monkey 1604 (lane 1), the monoclonal antibodies OKT4a (lane 2) and OKT4 (lane 4), and a negative control serum (lane 3).

TABLE 1

Immune Status before and Viral Activity of Rhesus Monkeys after the First Challenge with T-Cell-Grown SIVmac32H

Animal no.	SIV immunogene[a]	Immune status before challenge: NAB titers (Ig)[b]	Anti-gp130 titers (Ig)[c]	T-cell profile ration[d] (SI values)	CD4-binding inhibition (%)	Signs of infection after challenge: PCR	Virus isolation	Antibody response
Controls								
1721						+	+	Primary
1744						+	+	Primary
Vaccinees								
1698	gp130	3.7	5	—	70	−	−	—
1701	gp130	3.7	4	—	63	−	−	—
1715	gp130	4.0	5	—	94	−	−	—
1753	VVwt/gp130	1.9	3	4	5	+	+	Secondary
1763	VVwt/gp130	2.5	4	—	9	−	−	—
1729	VVgp130/gp130	2.2	6	8	49	+	−	—
1768	VVgp130/gp130	1.9	6	10	47	+	+	Secondary
1769	VVgp130/gp130	2.5	3	17	45	−	−	—

[a] gp130, virion-derived gp130; VVwt, vaccinia wild-type virus; VVgp130, vaccinia virus expressing SIV gp130.
[b] Nab, neutralizing serum antibodies measured in the MT-4 cell assay.
[c] Measured in ELISA using recombinant gp140 (Repligen) as antigen.
[d] rgp140 was used as antigen.

lated with strong CD4-binding inhibition determined as previously described (*27, 28*). All sera of the O-gp130 group inhibited the CD4 binding by more than 50%, whereas antisera from both VV-primed groups did not (Table 1). None of the immunized animals developed cytotoxic T-lymphocytes (CTL; data not shown) at any time. However, all VV-gp130-primed animals had developed a moderate to high proliferative T-cell response (*29*) with stimulation indices (SI) between 8 and 17, whereas only 1 of the 2 VV-wt-primed animals exhibited a low T-cell reactivity with a SI of about 4 (Table 1). The O-gp130 animals remained negative in this assay.

As measured by the gp130 ELISA after the challenge using gp130 oligomers as antigen (*14, 28*), all control animals developed a primary immune response indicating virus replication (Table 1). In contrast, none of the three O-gp130-immunized animals (1698, 1701, and 1715) exhibited a secondary immune response, indicating no SIVmac32H multiplication (Table 1). Respective results were obtained by virus reisolation and PCR analysis. Whereas all control animals became viremic and PCR positive, the O-gp130 animals remained negative (Table 1). Therefore the O-gp130 animals were protected against the infection with SIVmac32H. The VV-primed animals responded more heterogenously. Whereas one of the three VV-gp130 (1769)- and one of the two VV-wt-primed animals (1763) showed no sign of SIVmac32H infection and were thus protected, one of the VV-gp130 (1768) and one of the VV-wt animals (1753) developed a secondary antibody response and became viremic and PCR positive (Table 1). However, one of the VV-gp130 animals (1729) was only DNA–PCR positive but showed neither a secondary immune response nor viremia (Table 1), indicating suppression of virus replication. Analysis of the MHC class II alleles (*30*) of the animals revealed no association to protection (data not shown).

About half a year later, the protected animals were boosted twice 4 weeks apart with 100 μg O-gp130 per dose. Two weeks later, the revaccinated animals and three naive controls were challenged with 5 MID_{50} of the SIVmac32H/spI. At the time of challenge, all animals of the former O-gp130 group again developed high neutralizing antibody titers between 2.5×10^3 and 5×10^3, whereas both formerly VV-primed animals had developed intermediate neutralizing titers of about 0.6×10^3 (Table 2). gp130 binding titers ranged between 10^4 and 10^5. Unlike the response at the time of the first challenge, high neutralizing antibody titers did not correlate with an efficient serum CD4-binding inhibition (>50%, Table 2). Also in contrast to observations at the time of first challenge, at time of the second challenge only the animals of the former O-gp130 group had developed low to moderate proliferative T-cell responses with SI values of 3 to 7, whereas the animals from both former VV-primed groups remained negative (Table 2). After the second challenge all three control animals, 1513, 1623, and 1546, became infected. They exhibited a primary immune response and were positive in DNA–PCR (Table 2). However only two of the three control animals (1513 and 1623) became viremic. RNA–PCR was performed (*31*) to detect a low-level virus replication of the control animal 1546 negative in virus reisolation. All control animals

TABLE 2

Immune Status before and Viral Activity of Rhesus Monkeys after the Second Challenge with SIVmac32H/spI

Animal no.	SIV immunogen[a]	Immune status before challenge: Nab titers (Ig)[b]	anti-gp130 titers (Ig)[c]	T-cell profile ration[d] (SI values)	CD4-binding inhibition (%)	Signs of SIVmac32H infection after challenge: PCR Proviral DNA	PCR Genomic RNA	Plasma p24 antigen	Virus isolation	Antibody response
Controls										
1513						+	+	+	+	Primary
1546						+	+	−	−	Primary
1623						+	+	+	+	Primary
Vacinees										
1698	gp130	3.4	4	4	17	+	+	+	+	Secondary
1701	gp130	3.4	4	7	21	+	+	+	+	Secondary
1715	gp130	3.7	5	3	63	−	−	−	−	—
1763	VVwt/gp130	2.8	4	—	63	+	−	−	−	—
1769	VVgp130/gp130	2.8	4	—	60	−	−	−	−	—

[a] gp130, virion-derived gp130; VVwt, vaccinia wild-type virus; VVgp130, vaccinia virus expressing SIV gp130.
[b] Nab, neutralizing serum antibodies measured in the MT-4 cell assay.
[c] Measured in ELISA using recombinant gp140 (Repligen) as antigen.
[d] rgp140 was used as antigen.

were positive in RNA–PCR, indicating free virus particles in the plasma (Table 2). Two of the immunized animals from the former O-gp130 group (1698 and 1701) also had all signs of SIVmac infection. They exhibited a secondary immune response (Table 2) and were positive in virus reisolation and DNA– as well as RNA–PCR (Table 2). However, two of the immunized animals, no. 1715 of the former O-gp130 group and no. 1769 of the former VV-gp130 group, showed no secondary immune response and remained negative in virus isolation as well as DNA– and RNA–PCR (Table 2). These two animals were thus protected. The other animal from the former VV-wt group (1763) was only DNA–PCR positive but remained negative for all other infection parameters (Table 2), indicating suppression of virus replication. Again, a correlation of protection to MHC class II alleles could not be detected (data not shown).

Furthermore, RIPAs with extracts of ^{35}S-labeled SIVmac32H-infected C8166 cells were performed to investigate the serum reactivities against cellular components at the time of the first challenge. All immune sera precipitated in addition to gp130 a faint band of 45 kDa (Fig. 2A, lanes 1 to 3, and Fig. 2C, lanes 1 to 5). The latter band was not detected by the sera of the control animals (lanes 4 and 5). However, a similar faint band was also recognized by the MAB against HLA class I (Fig. 2A, lane 6, and Fig. 2C, lane 6) but not by all other MABs tested (Fig. 2A, lanes 7 to 8, and Fig. 2C, lanes 7 to 8). A very faint band of 45 kDa was also precipitated in addition to the gp130 by the antiserum of the SIVmac251-infected monkey 1604, but not by the negative monkey serum (Fig. 2B, lanes 1 to 2). At the time of the second challenge, the reactivity against cellular components of the immune sera, the MABs, as well as the antiserum from monkey 1604 was investigated by Western blot analysis with a pool of nonstimulated or stimulated PBMCs from different

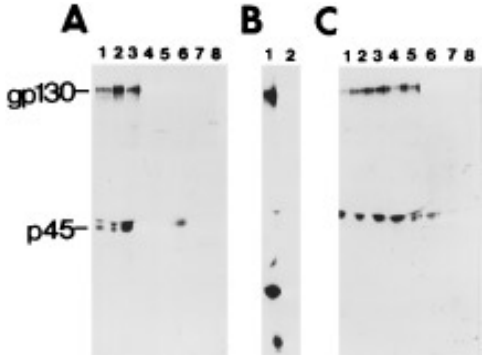

FIG. 2. Immunological reactivity of the immune sera at time of first challenge with a human T-cell line. C8166 cells, infected with SIVmac32H, were methabolically labeled and cell extracts were immunoprecipitated with the immune sera from the O-gp130 animals (A) 1698 (lane 1), 1701 (lane 2), and 1715 (lane 3), the control animals 1721 (lane 4) and 1744 (lane 5), and with the monoclonal antibodies against MHC class I (lane 6), MHC class II (lane 7), and OKT4 (lane 8). (B) As a positive and a negative control, immunoprecipitations were performed with the antiserum of monkey 1604, eprimentally infected with SIVmac251 (lane 1), and a serum from an uninfected animal (lane 2). (C) Similarly, immunoprecipitations were carried out with the immune sera of the VV-gp130 animals 1729 (lane 1), 1768 (lane 2), and 1769 (lane 3), of the VV-wt animals 1763 (lane 4) and 1753 (lane 5), and the monoclonal antibodies against MHC class I (lane 6), MHC class II (lane 7), and OKT4 (lane 8).

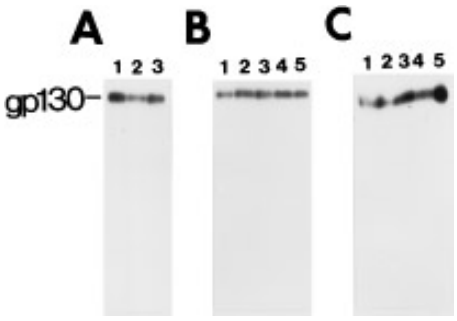

FIG. 3. Immunological reactivity of the immune sera at time of first and second challenge. Virion-derived gp130 oligomers were iodinated and immunoprecipitations were performed at the time of the first challenge (A) with the immune sera of the O-gp130 animals 1715 (lane 1), 1701 (lane 2), and 1768 (lane 3), (B) with the immune sera of the VV-gp130 animals 1729 (lane 1), 1753 (lane 2), and 1763 (lane 3), and the VV-wt animals 1768 (lane 4) and 1769 (lane 5) as well as at the time of the second challenge (C) with the immune sera 1698 (lane 1), 1701 (lane 2), 1715 (lane 3), 1763 (lane 4), and 1769 (lane 5). Immunoprecipitates were separated by SDS–PAGE and autoradiographed.

rhesus monkeys as well as with the spleen homogenate comprising the SIVmac32H/spI. In this test, a reactivity could not be detected (data not shown). Furthermore, all immune sera at time of the first (Figs. 3A and 3B) and second (Fig. 3C) challenges were tested for their reactivity with ^{125}I-labeled gp130 oligomers used for immunization. Only the gp130 was recognized.

The results presented above demonstrate that only the immunization with purified virion-derived gp130 oligomers could protect all rhesus monkeys against the homologous challenge with human T-cell-grown SIVmac32H. Priming with recombinant VV was not advantageous. The majority of animals was also protected against the challenge with the *ex vivo* isolate SIVmac32H/spI after revaccination of the protected animals half a year later. Thus, virion-derived purified envelope glycoproteins in an oligomeric form can be employed successfully as a subunit vaccine against the infection with SIVmac32H derived from cell culture or prepared *ex vivo.* In the SIV macaque model, a similar complete protection was only obtained with whole inactivated virus (*8*) but not with subunit vaccines based on recombinant or virion-derived external glycoproteins in a monomeric form. Only a partial protection was achieved with an enriched, virion-derived monomeric gp130 preparation (*15*), but not with recombinant gp130- or gp160-based subunit vaccines (*16, 17*). Only one group reported protection of rhesus monkeys against an SIV challenge (*20*). These animals were primed with VV expressing the SIV glycoprotein gp160 followed by immunization with partially purified gp160 expressed in insect cells. However, other authors could not confirm this result using a similar immunization schedule (*16, 32*). Recently, different subunit vaccines based on recombinant monomeric gp130 or gp160 molecules were tested (*18, 19*). Again, in no case could protection be obtained. Therefore, recombinant monomeric gp130 or gp160 preparations are apparently unsuitable as vaccines against SIV. This conclusion was also confirmed by another recent study comparing whole inactivated viral vaccines with different recombinant subunit preparations (*33*). In this study four levels of resistance to SIV infection were obtained: (i) no detectable virus (sterilizing immunity), (ii) abortive infection (strong immunity) characterized by virus or provirus detection early after challenge, (iii) suppression of infection (incomplete or partial immunity) defined by a terminated viremia, declining of antibody titers after challenge and low provirus load, or (iv) active infection (no immunity). Sterilizing immunity was only obtained with whole inactivated virus. A strong immunity was induced only in two animals, one immunized with VV-env and one immunized with VV-gag-env. The majority of the animals had developed an incomplete or partial immunity and VV priming was not advantageous.

This is in accordance to our results, where VV priming was also not superior. Whereas all our O-gp130-immunized animals had developed a sterilizing immunity at the time of the first challenge, only one of the three animals of the VV-gp130 group and one of the two animals of the VV-wt group were completely immune. However, in two animals, one of the VV-gp130 group and one of the VV-wt group, an immune status not described earlier in the SIV system was observed. Both animals were positive only in the DNA–PCR, but negative for all other infection parameters. Obviously, the primary infection of these animals could not be prevented, but later virus replication was effectively suppressed. In an earlier vaccine trial with HIV-2, a similar situation was found (*14*). We suggest to designate this status as silent infection. At the time of the second challenge two of the five animals had developed a sterilizing immunity and one of the five animals a silent infection. Thus, the gp130 vaccine had also induced a protective immunity against the *ex vivo* isolate SIVmac32H/spI in three of five animals.

Until now, a protective immunity could not be induced with SIV vaccines produced on human T-cell lines against a challenge with cell-free SIVmac251 propagated on monkey PBMC (*8*). This phenomenon was explained with xenoimmunization effects against cellular proteins derived from human T-cells (*34*). Our results demonstrated that at the time of the first challenge all immune sera recognized in addition to the gp130 a 45-kDa protein in SIVmac32H-infected C8166 cells. This 45-kDa protein also reacted with a MAB against HLA class I, but also with the antiserum of SIVmac251-infected rhesus monkey 1604. Thus, our gp130 oligomers might be contaminated with traces of HLA class I molecules present in virions of SIVmac and HIV-1 (*35*). However, neither the immune sera nor the MABs recognized such a 45-kDa

protein in the preparations of the gp130 oligomers used for immunization. Perhaps the gp130 molecule shares epitopes with the 45-kDa protein, leading to this cross-reactivity. Therefore, a protection induced by xenoimmunization does not appear to play a major role. A molecular mimicry might explain our serological findings. In individuals vaccinated with gp120 of HIV-1, gp120/HLA class I cross-reactive antisera were defined (*36*). However, at the time of the second challenge, all immune sera and the MAB did not react with proteins of nonstimulated or stimulated PBMC from rhesus monkeys and the spleen extract containing the SIVmac32H/spI. Therefore, alloimmunization effects can be ruled out and a molecular mimicry between the gp130 and the MHC molecules present on rhesus monkey PBMC or in the SIVmac32H/spI preparation was also unlikely.

In several immunization studies protection could neither be correlated to a specific humoral nor cellular immune reactivity (*5, 6*). Only in one case, the protective immunity coincided with a proliferative T-cell response (*29*). In the present investigation, at the time of the first challenge, a clear correlation of protection to high neutralizing antibody titers was found in the O-gp130 group. A similar correlation was also found in the VV-gp130 or VV-wt group. Therefore, the development of intermediate to high neutralizing antibodies is apparently essential for protection against a primary infection. The protective neutralizing mechanism seemed to be based on the CD4-binding efficacy. Interestingly, a similar correlation of protection to neutralizing antibody titers was not obtained after the second challenge. The immune mechanism protecting against the second challenge is obviously distinct from that suppressing viral replication after the first challenge. This type of immune response appears to be a consequence of the first challenge.

In conclusion, the present vaccine experiment demonstrated that a protective immunity against the challenge with human T-cell-grown SIVmac32H or SIVmac32H/spI prepared *ex vivo* can be induced by immunization with virion-derived gp130 oligomers. The observed protection was correlated with the induction of neutralizing and/or CD4-binding inhibition antibodies, but not to proliferating T-cells. A xeno- or alloimmunization effect appears unlikely. Priming with VV does not seem to be advantageous. Thus, immunization with the external glycoprotein gp130 of SIVmac32H in an oligomeric form can induce a protective immunity against challenge with cell-free virus either grown on human T-cells or prepared *ex vivo.*

ACKNOWLEDGMENTS

We thank Dr. A. Burny, Free University of Brussels, Belgium, for providing the recombinant SIV gp130 expressing vaccinia virus. We acknowledge S. Bartels, K. Borchard, A. Schäfer, and K. Wase for their skillful technical assistance and thank K. Peinemann for typing the manuscript and preparing the tables. This work was supported by a grant of the Bundesgesundheitsamt, Berlin, FRG, the EU Programme EVA, and the EU Concerted Action "Monkey Models for AIDS Research."

REFERENCES

1. Alter, H. J., Eichberg, J. W., Masur, H., Saxinger, W. C., Gallo, R., Macher, A. M., Lane, H. C., and Fauci, A. S., *Science* 226, 549–552 (1984).
2. Daniel, M. D., Letvin, N. L., King, N. W., Kannagi, M., Seghal, P. K., Kanki, P. K., Essex, M., and Desrosiers, R. C., *Science* 228, 1200–1204 (1985).
3. Stahl-Hennig, C., Herchenröder, O., Nick, S., Evers, M., Stille-Siegener, M., Jentsch, K. D., Kirchhoff, F., Tolle, T., Gatesman, T., Lüke, W., and Hunsmann, G., *AIDS* 4, 611–617 (1990).
4. Letvin, N., Daniel, M., Sehgal, P., Desrosiers, R., Hunt, R., Waldron, L., Mackey, J., Schmidt, D., Chalifoux, L., and King, N., *Science* 225, 840–842 (1985).
5. Murphey-Corb, M., Martin, L. N., Davison-Fairburn, B., Montelaro, R., Miller, M., West, M., Ohkawa, S., Baskin, G. B., Zhang, J. Y., Putney, S., Allison, A. C., and Eppstein, D., *Science* 246, 1293–1297 (1989).
6. Carlson, J. R., McGraw, T. P., Keddie, E., Yee, J. L., Rosenthal, A., Langlois, A. J., Dickover, R., Donovan, R., Luciw, P. A., Jennings, M., and Gardner, M. B., *AIDS Res. Hum. Retroviruses* 6, 1239–1246 (1990).
7. Johnson, P. R., Montefiori, D. C., Goldstein, S., Hamm, T. E., Zhou, J., Kitov, S., Haigwood, N. L., Misher, L., London, W. T., Gerin, J. L., Allison, A., Purcell, R. M., Chanock, R. H., and Hirsch, V. M., *Proc. Natl. Acad. Sci. USA* 89, 2175–2179 (1992).
8. The European Concerted Action on "Macaque Models for AIDS Research," *Vaccine* 13, 295–300 (1995).
9. Niedrig, M., Gregersen, J., Fultz, P., Broker, M., Mehdi, S., and Hilfenhaus, J., *Vaccine* 11, 67–74 (1993).
10. Arthur, L. O., Bess, J. W., Waters, D. J., Pyle, S. W., Kelliher, J. C., Nara, P., Krohn, K., Robey, W. G., Langlois, A. J., Gallo, R. C., and Fischinger, P. J., *J. Virol.* 63, 5046–5053 (1989).
11. Berman, P. W., Groopman, J. E., Gregory, T., Clapham, P. R., Weiss, R. A., Ferriani, R., Riddle, L., Shimasaki, C., Lucas, C., Lasky, L. A., and Eichberg, J., *Proc. Natl. Acad. Sci. USA* 85, 5200–5204 (1988).
12. Berman, P. W., Gregory, T. J., Riddle, L., Nakamura, G. R., Champe, M. A., Porter, J. P., Wurm, F., Hershberg, R. D., Cobb, E. K., and Eichberg, J., *Nature* 345, 622–625 (1990).
13. Girard, M., Kieny, M. P., Pinter, A., Barre-Sinoussi, F., Nara, P., Kolbe, H., Kusumi, K., Chaput, A., Reinhart, T., Muchmore, E., Ronco, J., Kaczorek, M., Gomard, E., Gluckman, J. C., and Fultz, P. N., *Proc. Natl. Acad. Sci. USA* 88, 542–546 (1991).
14. Lüke, W., Voss, G., Stahl-Hennig, C., Coulibaly, C., Petry, H., and Hunsmann, G., *AIDS Res. Hum. Retroviruses* 9, 387–394 (1993a).
15. Murphy-Corb, M., Montelaro, R., Miller, M., West, M., Martin, L., Davison-Fairburn, B., Ohkawa, S., Baskin, G., Zhang, J., Miller, G., Putney, S., Allison, A., and Eppstein, D., *AIDS* 5, 655–662 (1991).
16. Giavedoni, L., Planelles, V., Haighwood, N., Ahmad, S., Klug, J., Marthas, M., Gardner, M., Luciw, P., and Yilma, T., *J. Virol.* 67, 577–583 (1993).
17. Mills, K., Page, M., Chan, W., Kitchin, P., Stott, J., Taffs, F., Jones, W., Rose, J., Ling, C., Silvera, P., Corcoran, T., Flanagan, B., Burny, A., Bex, F., Delchambre, M., VanOpstal, O., Fabry, L., Thiriart, C., Delers, A., DeWilde, M., and Bruck, C., *J. Med. Primatol.* 21, 50–57 (1992).
18. Ahmad, S., Lohman, B., Marthas, M., Giavedoni, L., El-Ahmad, Z., Haighwood, N., Scandella, C., Gardner, M., Luciw, P., and Yilma, T., *AIDS Res. Hum. Retroviruses* 10, 195–204 (1994).

19. Ohkawa, S., Wilson, L., Larosa, G., Javaherian, K., Martin, L., and Murphey-Corb, M., *AIDS Res. Hum. Retroviruses* 10, 27–38 (1994).
20. Hu, S.-L., Abrams, K., Barber, G. N., Moran, P., Zarling, J. M., Langlois, A. J., Kuller, L., Mortan, W. R., and Benveniste, R. E., *Science* 255, 456–459 (1992).
21. Lüke, W., Stahl-Hennig, C., Nick, S., Voss, G., Petry, H., Coulibaly, C., Fuchs, D., Wachter, H., and Hunsmann, G., *In* "Vaccines 92: Modern Approaches to New Vaccines Including Prevention of AIDS" (Ginsberg, Brown, Lerner, and Chanock, Eds.), pp. 103–108. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1992.
22. Bachmann, B., Lüke, W., and Hunsmann, G., *Nucleic Acids Res.* 18, 1309 (1990).
23. Stahl-Hennig, C., Voss, G., Nick, N., Petry, H., Fuchs, D., Wachter, H., Coulibaly, C., Lüke, W., and Hunsmann, G., *Virology* 186, 588–596 (1992).
24. Stahl-Hennig, C., Voss, G., Dittmer, U., Coulibaly, C., Petry, H., Lüke, W., and Hunsmann, G., *AIDS* 7, 1–9 (1993).
25. Voss, G., Dittmer, U., Coulibaly, C., Makoschey, B., Petry, H., Lüke, W., and Hunsmann, G., *J. Gen. Virol.* 74, 1757–1763 (1993).
26. Harada, S., Purtilo, D., Koyangi, Y., Sonnabend, J., and Yamamoto, N., *J. Immunol. Methods* 92, 177–181 (1986).
27. Polzien, F., Scharf, J., Lüke, W., and Hunsmann, G., *AIDS Res. Hum. Retroviruses* 8, 1171–1177 (1992).
28. Lüke, W., Polzien, F., Scharf, J. G., and Hunsmann, G., *J. Virol. Methods* 42, 169–180 (1993).
29. Voss, G., Nick, S., Stahl-Hennig, C., Coulibaly, C., Petry, H., Lüke, W., and Hunsmann, G., *J. Gen. Virol.* 73, 2273–2281 (1992).
30. Christ, R., Sauermann, U., and Hunsmann, G., *Tissue Antigens* 44, 241–247 (1994).
31. Petry, H., Dittmer, U., Stahl-Hennig, C., Coulibaly, C., Makoschey, B., Fuchs, D., Wachter, H., Kaup, F., Jurkiewicz, E., Lüke, W., and Hunsmann, G., *J. Virol.* 69, 1564–1574 (1995).
32. Mills, K., Page, M., Kitchin, P., Chan, L., Jones, W., Silvera, P., Corcoran, T., Flanagan, C., Ling, C., Thiriart, C., DeWilde, M., Bruck, C., Rud, E., Clark, B., and Stott, J., *J. Med. Primatol.* 22, 104–109 (1993).
33. Israel, Z., Edmonson, P., Maul, D., O'Neil, S., Mossman, S., Thiriart, C., Fabry, L., VanOpstal, O., Bruck, C., Bex, F., Burny, A., Fultz, P., Mullins, J., and Hoover, E., *J. Virol.* 68, 1843–1853 (1994).
34. Stott, E. J., *Nature* 353, 393 (1991).
35. Arthur, L., Bess, J., Sowder, R., Benveniste, R., Mann, D., Chermann, J., and Henderson, L., *Science* 258, 1935–1938 (1992).
36. De-Santis, C., Lopalco, L., Robbioni, P., Longhi, R., Rappocciolo, G., Siccardi, A., and Beretta, A., *AIDS Res. Hum. Retroviruses* 10, 157–162 (1994).